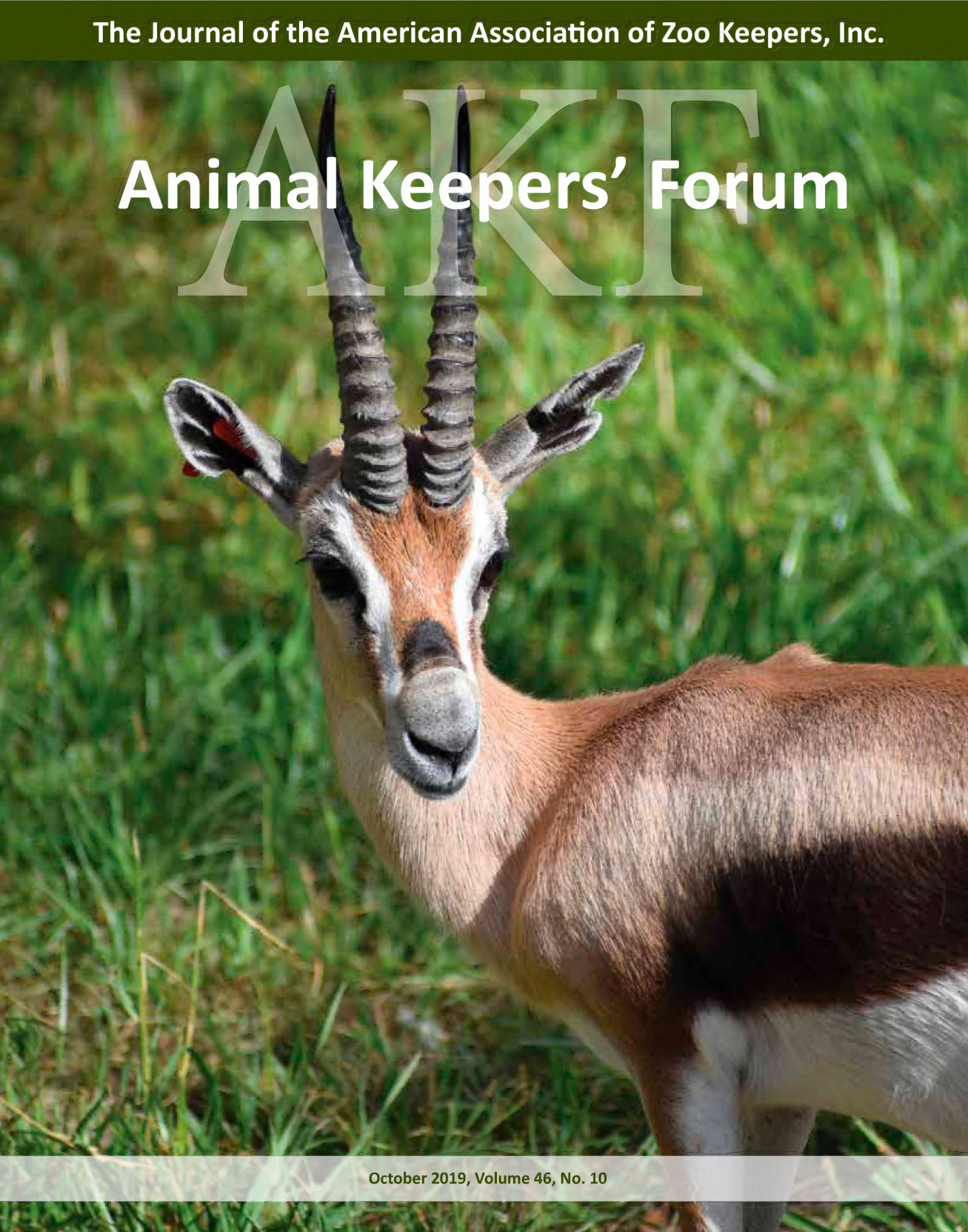
The Journal of the American Association of Zoo Keepers, Inc.

Animal Keepers' Forum



October 2019, Volume 46, No. 10

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MISSION STATEMENT

American Association of Zoo Keepers, Inc.

The American Association of Zoo Keepers, Inc. exists to advance excellence in the animal keeping profession, foster effective communication beneficial to animal care, support deserving conservation projects, and promote the preservation of our natural resources and animal life.

ABOUT THE COVER

This month's cover photo comes to us from Elena Bell of the Akron Zoological Park and features a Speke's Gazelle (*Gazella spekei*). Speke's gazelles are native to the Horn of Africa, specifically Somalia. They received their name from an English explorer, John Hanning Speke, who is famous for discovering the source of the Nile River in 1858. On average, they are two feet tall (at the shoulder) and weigh 25-40 pounds. They are the smallest of all gazelle species.

Speke's gazelles have s-shaped horns, with the male being larger than the female. The majority of their fur is tan, with a dark band down their side and a white stripe on their nose and belly. The gazelles have an interesting-looking nose. There is loose skin on the top of their nose that they can inflate, which amplifies their honk-like alarm calls.

Speke's Gazelles are classified as an Endangered species. The gazelle population is decreasing due to loss of habitat, poaching and overgrazing by livestock, such as cattle and goats. Their population numbers have decreased by 50% since 1988. There are currently no protected areas within the range of Speke's gazelles. The conservation status of this species is expected to decline further in the absence of protection and management of wild populations and their habitat.

Articles sent to *Animal Keepers' Forum* will be reviewed by the editorial staff for publication. Articles of a research or technical nature will be submitted to one or more of the zoo professionals who serve as referees for *AKF*. No commitment is made to the author, but an effort will be made to publish articles as soon as possible. Lengthy articles may be separated into monthly installments at the discretion of the Editor. The Editor reserves the right to edit material without consultation unless approval is requested in writing by the author. Materials submitted will not be returned unless accompanied by a stamped, self-addressed, appropriately-sized envelope. Telephone, fax or e-mail contributions of late-breaking news or last-minute insertions are accepted as space allows. Phone (330) 483-1104; FAX (330) 483-1444; e-mail is shane.good@aazk.org. If you have questions about submission guidelines, please contact the Editor. Submission guidelines are also found at: aazk.org/akf-submission-guidelines/.

Deadline for each regular issue is the 3rd of the preceding month. Dedicated issues may have separate deadline dates and will be noted by the Editor.

Articles printed do not necessarily reflect the opinions of the *AKF* staff or the American Association of Zoo Keepers, Inc. Publication does not indicate endorsement by the Association.

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ANIMAL KEEPERS' FORUM

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As a member of the Board of Directors, I find myself thinking of the year as running from one AAZK Annual Conference to the next. I always have that week circled on the calendar as a highlight of my year both personally and professionally. The 2019 AAZK Annual Conference was no exception! The Indianapolis AAZK Chapter and Indianapolis Zoo were fantastic hosts and the outstanding effort that went into the planning was evident to everyone in attendance. While each Annual Conference is a transitional period, 2019 was especially unique for me as it featured a great deal of exciting beginnings and bittersweet endings.

The end of the conference is always sad as I bid farewell to professionals and friends both old and new, but bidding farewell to three outgoing Board members, Bethany Bingham, Mary Ann Cisneros, and Bill Steele, was especially difficult. These three individuals served as my mentors on the Board as they were the three continuing members when I first joined at the 2017 Conference in DC. It was a pleasure to work with them as we sought to lead the organization and I will miss working with them as leaders.

The completing terms of three Board members does bring with it the excitement of three new Board members coming aboard. James Weinpress, Kristen Scaglione, and Abbie Doan were sworn in at the Closing Banquet and will join Ellen Vossekuil and Nicole Pepo to form the AAZK Board of Directors. Nicole Pepo will also be taking on a change herself as she moves into the role of Vice President vacated by Mary Ann. Finally, I stepped into Bethany Bingham's role as the new President of AAZK. This organization has meant a great deal to me throughout my career and I look forward to working with Nicole as the Executive Officers and the Board of Directors new and continuing as we strive to serve the membership and uphold the standards of the organization to the best of our ability.

Transition can be challenging, and moving on from the excitement of the AAZK Annual Conference knowing that you have to wait a full year to connect with your peers again is especially tough. However, the prospects for personal, professional, and organizational growth that I hope every conference attendee carries away always drives me through until the following year. So until I see so many of you next year in Los Angeles for the 2020 AAZK Annual Conference, thank you for a great week and "Happy New Year" to all!

Cheers,

Paul Brandenburger
Paul.Brandenburger@AAZK.org

COMING EVENTS

Post upcoming events here!
e-mail shane.good@aazk.org

November 1-4, 2019
Canid and Hyenid Husbandry Course
Glen Rose, TX
Hosted by Fossil Rim Wildlife Center
For More information contact: hgenter@denverzoo.org

November 4-7, 2019
Polar Bear Workshop
Toronto, Ontario, Canada
Hosted by Toronto Zoo
For more information go to: education.torontozoo.com/products/1099943-polar-bear-workshop-2019.aspx

November 13-15, 2019
Turtle and Tortoise Preservation Group Conference
Mesa, AZ
For more information go to: ttpg.org/conferences.php

November 15-18, 2019
ZAA 14th Annual Conference
Montgomery, AL
Hosted by Alabama Safari Park
For more information go to: zaa.org

January 8-11, 2020
15th North American Crane Workshop
Lubbock, TX
For more information go to: nacwg.org/workshop15.html

January 14-16, 2020
5th Annual Animal Training Workshop
San Antonio, TX
Hosted by San Antonio Zoo
For more information go to: sazoo.org/trainingworkshop/

March 4-7, 2020
Venom Week 2020
Gainesville, FL
Hosted by The North American Society of Toxicology
For more information go to: reg.conferences.dce.ufl.edu/VENOM/1566

April 4-9, 2020
AZA Mid-Year Meeting
Palm Springs, CA
Hosted by The Living Desert Zoo and Gardens.
For more information go to: aza.org/conferences-meetings

June 22-26, 2020
Zoos and Aquariums Committing to Conservation
Salt Lake City, UT
Hosted by Utah's Hogle Zoo and Tracy Aviary
For more information go to: zaccconference.com/

AMERICAN ASSOCIATION of ZOO KEEPERS

August 30 - September 3 2020

AAZK National Conference
Los Angeles, CA
Hosted by Los Angeles AAZK Chapter and Los Angeles Zoo

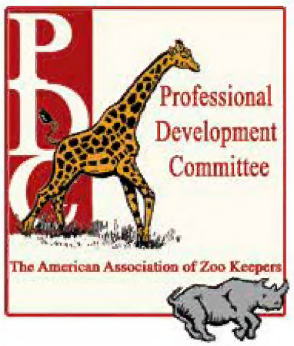
www.aazk2020.org/

September 13-17, 2020
AZA Annual Conference
Columbus, OH
Hosted by the Columbus Zoo and Aquarium
For more information go to: aza.org/conferences-meetings

NEW DIRECTOR OF PROFESSIONAL DEVELOPMENT AND CONFERENCE MANAGEMENT NAMED

AAZK has completed the employment search for the AAZK Director of Professional Development and Conference Management (PDCM). The application process was distributed to the membership of AAZK via publication in AKF, AAZK Social Media and AAZK Job Board.

After independent review of the submitted applications by a panel of external professionals contracted for the process, AAZK has followed the recommendations of the panel and is pleased to announce the hire of Bethany Bingham as the new Director of AAZK PDCM.



AAZK Professional Development Committee Now Accepting Topical Workshop Applications for the 2020 AAZK National Conference

The 46th Annual AAZK National Conference
Los Angeles, California
August 30 – September 3, 2020
Conference Theme: "Lights, Camera… Take Action!"

The AAZK Professional Development Committee is pleased to announce the call for Topical Workshops for the 2020 AAZK National Conference hosted by the Los Angeles Chapter of AAZK.

Deadline for Submission of Abstracts for Workshops: January 15, 2020
Authors will be notified regarding acceptance no later than **February 15, 2020.**

Workshops Format
Workshop subjects should be in-depth explorations of animal health, animal management, taxa-specific husbandry, conservation, and keeper professional development. Workshops should be two hours in length. Subjects that require more than two hours should be submitted as "Part One" and "Part Two". Abstracts should be no more than 250 words and should focus on the main theme of the Workshop.

Open Topical Workshops
The Open Workshop format will offer unlimited attendance (based on the capacity of the ballroom) and will be best suited for lecture-based workshops with a Q & A session at the end.

Limited Topical Workshops
Held in limited capacity breakout rooms, this format is best suited for small group interactive workshops and will have a cap on the number of participants.

How to Submit Your Abstract for Consideration:
Follow this link to fill out our Google Form Application: **https://docs.google.com/forms/d/1pHyY8uGq_ZakHyF9Os00X-bzXiZDwjcyBuQdHaMqjWA/viewform?edit_requested=true**

You may also email PDC@aazk.org for a direct link to the Google Form, or visit the conference website for more information at **https://www.aazk2020.org/**

Any questions should be directed to **PDC@aazk.org** with ATTN: Topical Workshop as part of the email subject.

Hypothesis in Testing: Early Diagnosis of EEHV Infection via Retinal Scans

Emma Wigdahl, Senior Zoo Keeper
Albuquerque BioPark, Albuquerque, New Mexico

Michelle A. Ozbun, Professor
Department of Molecular Genetics & Microbiology,
The University of New Mexico School of Medicine, Albuquerque, New Mexico

Abstract

At the Albuquerque BioPark we have a theory that highly pathogenic Elephant Endotheliotropic Herpesvirus (EEHV) infections can be detected through retinal imaging. To this end we have performed preliminary testing with a handheld ophthalmic camera (Volk® Pictor Plus). Using this device we successfully image the retinas of Jazmine, an Asian elephant (*Elephas maximus*), on a daily basis with clear images of her optic disc and blood vessels. We are testing the hypothesis that the virus infection may present in the early stages via micro-aneurysms and/or hemorrhages that could be detected in vivo with the camera; such information would be an indication to staff to immediately draw blood and perform polymerase chain reaction (PCR) for detection of EEHV DNA and straightaway start treatment.

Introduction

Elephant Endotheliotropic Herpesvirus (EEHV) causes an often deadly viral hemorrhagic disease that progresses rapidly, usually in Asian elephants. This virus is carried by wild elephants and elephants in human care, and while usually dormant, it can reactivate, be shed to naïve (typically juvenile) animals and cause widespread disease. It is not known exactly how the virus spreads, what causes it to wake, and there is no proven treatment, vaccination, or cure. Like many diseases, blood draws are the reliable method for detection. Yet as the industry standard is weekly testing, which can miss very early detection, we saw a need for a daily non-invasive option with real-time results. As most animal keepers know maintaining a blood draw behavior can be challenging; with a weekly stick, this is especially true. Since the test is usually done on young elephants ages

Image 1 Diseased human retina

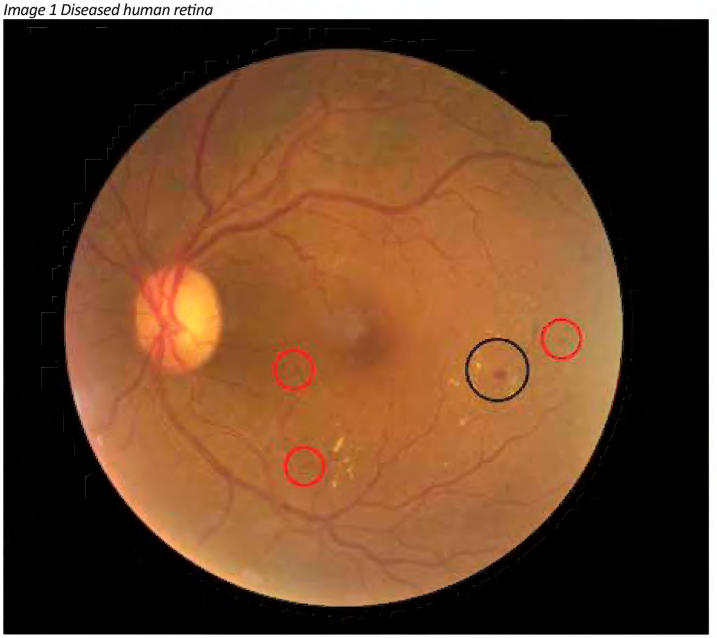
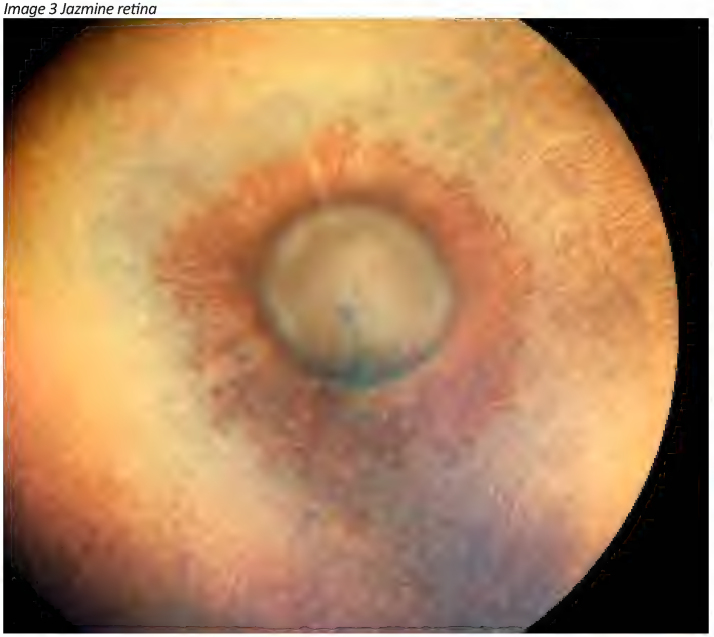


Image 2 Jazmine eye scan

1-8 years old the behavior training can be downright daunting. Absent regular testing can mean missing the early, non-symptomatic stages of the virus infection when supportive care is thought to be most beneficial. Unfortunately, even weekly testing can fail to be frequent enough with elephants becoming ill and succumbing during that timeframe. Due to these challenges, it became obvious to our BioPark team that new methods for EEHV infection detection were needed to save these animals. We became aware of retinal imaging for medical diagnosis in humans and discovered that it had the potential to provide daily evaluation in a painless approach that can be performed entirely in-house by keeper staff. We do not foresee this method as a replacement for PCR bloodwork, but propose retinal scanning as a complement to DNA detection that might fill the screening gap between each draw.

Image 3 Jazmine retina



The rationale for testing the retinal imaging is based on a number of scientific findings. Research suggests the deadly EEHV strains begin infection in the elephant trunk, spreading through the body, and without adequate intervention generally ends in a fatal coronary episode (Stanton et al., 2010). The virus attacks endothelial cells which line the inside of blood vessels in the body. Studies have shown intermittent shedding of EEHV in ocular secretions in healthy elephants, which may indicate low-level disease in the eye (Stanton et al., 2010; Hardman et al., 2012). There are cases of ocular manifestations detected during both hemorrhagic and non-hemorrhagic virus infections (Bacsal et al., 2007; Chan et al., 2006; Cruz-Villegas et al., 2003; Kapoor et al., 2006; Lim et al., 2004; Su et al., 2007). Of particular interest is that ocular changes have been found in cases of human herpesvirus infections (Takase et al., 2014).

Our theory is that when the EEHV attacks the endothelial cells, there will be visual evidence of the damage in the retinal blood vessels. The eye is one of the only places in the body that blood vessels can be imaged in vivo. Signs of distress would likely present as blood leakage (micro-aneurysms and/or hemorrhages) that would be readily visualized and captured on the retinal images (Image 1). Our hope is that observing these signs, or lack thereof, will aide in the early diagnosis of EEHV and help fill the diagnostic gaps between weekly or biweekly blood draws. This approach is a painless, simple daily routine that may have a major impact on elephant health management and EEHV infection diagnosis and care. The camera we use is a handheld, lightweight, retinal imaging device that easily connects to a computer so users can immediately grade the images. With

a little training the camera is easy to use and the imaging process takes less than five minutes to complete.

Methods

At the Albuquerque BioPark we have 2.4 Asian elephants, but only one (Jazmine, 4 years old) is in the traditional age range for contracting a lethal EEHV infection, so she is the only animal we regularly test. Every morning Jazmine enters the training chute and presents her eyes through a gap in the training wall (Image 2). We image both eyes and after Jazmine returns to the stall her keeper grades the images before the elephants go on exhibit for the day. Thus far, we have established an extensive baseline of normal retinal images for Jazmine, and no significant changes have been noted (Image 3). Yet, if we were to obtain deviant, suspect images, procedures are in place to immediately draw blood and preform a PCR test for EEHV (at the University of New Mexico); concomitantly, we would start antiviral and supportive care. Overall, the entire imaging procedure takes about five minutes and fills in the gap between weekly blood draws.

Discussion

We would like to make clear that we are still in the grant funding stage for this project and this is not a formal research proposal. Despite this, we feel that the lives of elephant calves are too important to keep this project to ourselves any longer, and want to share it with the zoo community and get other facilities involved. Retinal imaging can provide facilities struggling to get weekly blood, who experience the occasional missed blood test, or who want a daily test, another EEHV screening option.

This is a cutting edge, novel approach with potential for detecting EEHV infection at an early stage. To our knowledge no others have pursued retinal imaging as a means of finding early signs of this often lethal disease. Nevertheless, as we have not had a detectable EEHV outbreak since beginning this project one year ago, we lack data that would either support or refute our hypothesis. It would greatly benefit this research endeavor if other facilities with elephant calves would implement this testing procedure by obtaining a retinal imaging camera and training their calves to accept daily photos. We are happy to freely consult in training for the use of the camera and for grading images. As retinal imaging is often used in humans to detect early signs of diabetes and heart disease, we believe these approaches may have applications for the health care of other animals in a zoological facility. If your facility is interested in our project and would like more information, please contact the Albuquerque BioPark Elephant Manager Rhonda Saiers at rsaiers@cabq.gov

As retinal imaging is often used in humans to detect early signs of diabetes and heart disease, we believe these approaches may have applications for the health care of other animals in a zoological facility.

Acknowledgments

We are extremely grateful to Jeff Wigdahl Ph.D. and Vision Quest Biomedical for their assistance, advice, and encouragement, and for providing the equipment in the early stages of this research. An additional thank you goes out to the Albuquerque BioPark for their support, and to the BioPark Society for funding this research and making it possible.

The authors have no affiliation with Volk Optical Inc., and this company had no role in this research program or the writing of this manuscript.

Equipment

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A Systematic Review and Comparison of Change in Fecal Stress Markers Among Captive Non-human Primates, Predators, and Herbivores

Jennifer Hooson BS, CVT, Graduate Student
Miami University
Redmond, Oregon

Abstract

Researchers have recognized that captive animals experience stress and that stress can be detrimental to the animals. The negative effects of stress highlighted in this report are derived from a continual stimulation of the hypothalamic-pituitary-adrenal axis. The result is a cascade effect that leads to the depletion of glucose stores, the suppression of the immune system, and a decrease in reproductive functions. Glucocorticoids, a by-product of this cascade, has been measured in various mediums (serum, plasma, urine, saliva, and feces) collected from animals. By comparing these measured by-products of stress-related hormones, researchers can determine when an animal is reacting to stress and if measures are taken to mitigate that stress is working. The two purposes of this research were to show, by way of average fold change in these markers (the change measured divided by the baseline or normal level prior to the stressor), whether or not there was a difference between i) non-human primates (NHP), mammalian predators, and mammalian herbivores in their stress levels in captivity and ii) if the animals are reacting more to anthropogenic, inter/intra animal, or other stressors. To obtain this information, peer-reviewed studies which recorded their findings of the fecal stress markers were analyzed and the fold change calculation was applied to the data. The results suggested that there is no significant difference in stress levels between the partial clades. However, a significant difference was found between the stressor categories. Based on these results it is recommended that further studies be conducted across the clades and stressor categories to determine if these findings can be generalized. The results of this study could help captive animal managers focus on those animals most susceptible to stress and minimize the stimuli that are causing the most stress.

Introduction

Captive animals hold our imaginations and the hope of their species. Zoos and various captivity facilities are charged with maintaining accomplishing these two mighty responsibilities. The call to protect healthy gene pools of quickly declining wild animals due to a variety of causes has been answered by many zoos (Caldwell, 2014; Tribe and Booth, 2003). The ultimate goal of such programs is to maintain a sustainable gene pool, so that one-day endangered species may be reintroduced to the wild (EAZA, 2016; Tribe and Booth, 2003). Researchers can aid in the management of these programs by helping to better understand the species being cared for. When learning more about a species, while simultaneously employing the best management practices, a concept called Active Adaptive Management is utilized (McCarthy & Possingham, 2007). In this process, researchers are constantly re-evaluating their methods and the impact of those methods on the management of the species in question. One component of management that will be focused on for the sake of this paper is the monitoring of an animal's stress and possible distress.

Stress is a biological response to a threat to homeostasis, which could be indicated by an increase in concentrations of adrenal steroids like glucocorticoids or their excreted metabolites, called corticoids, circulating in the blood (Linklater et al., 2010). Distress would then be defined as a chronic condition resulting from repeated and/or cumulative stress. Distress is differentiated from

stress by its biological cost due to the rerouting of resources from core survival and reproduction functions. It can be measured by a spike in metabolites (stress response) and then a drop in these same metabolites to a point that is lower than the baseline level (adrenal suppression; Linklater et al., 2010).

Often times, animals in captivity are observed displaying the behavioral manifestations of stress, such as pacing, lethargy, and other stereotypic behaviors (Chosy et al., 2014). In some cases, there are less observable long-term effects of chronic stress (a.k.a distress), including weight loss, illness, infertility, and sometimes even death (Jacobs et al., 2014, von der Ohe & Servheen, 2002). What many visitors may not know is that it is not necessarily being in captivity that is causing the animals to experience stress, but instead the circumstances or situations they encounter while in captivity. Potential stressors range from introduction/removal from conspecifics, construction that is happening on their habitat, exposure to visitors, or a new enrichment activity, among others (Chosy et al., 2014; Jacobs et al., 2014 ; Li et al., 2007; Liu et al., 2006 ;Rafac & Santymire, 2013; Schell et al., 2013; Webster et al., 2016; Wielebnowski et al., 2002; Zaragoza et al., 2011). For the health and sustainability of these species, it is imperative that we, as stewards of these animals, find ways to minimize the impacts of negative stimuli.

One method used to monitor stressors and stress is to follow hormonal stress markers called fecal glucocorticoid metabolites (FGMs) (Schell et al., 2016; von der Ohe & Servheen, 2002). Glucocorticoids (i.e., cortisol and corticosterone) are steroid hormones that become elevated in response to stressors (Stevenson et al., 2018). There are a wide variety of biological substances created by the animal from which researchers could extract these steroid hormones. However, the hormones that are excreted via feces have been shown to represent an average concentration of these hormones over a given time period that is associated with normal digestive excretion times (Jacobs et al., 2014). The collection of the fresh feces by animal care staff has been shown to have little to no effect on the animals, therefore reducing the chances of having an artificially elevated glucocorticoid concentration from the collection of the sample (Jacobs et al., 2014; von der Ohe & Servheen, 2002).

To help validate the concentrations that the researchers find in the feces, they will often run what is called an Adrenocorticotropic Hormone (ACTH) challenge to stimulate the Hypothalamic-Pituitary-Adrenal Axis (HPA axis) in order to physiologically validate their FGM assays (Schell et al., 2013). This is done by injecting the animal intravenously with ACTH, which stimulates the HPA axis to respond like it is being stimulated by stress (Scheidegger et al., 2016). The HPA axis then releases the glucocorticoid metabolites which are then excreted in the feces. This process gives researchers a baseline response to stress for a species, which they can then use to compare results from other stressors (Scheidegger et al., 2016). It also is a way for researchers to prove their testing for the FGMs works for a species because they know ACTH will cause a response.

Through the work of many researchers and captive animal facilities, there is a growing database of stress values in different animal partial clades (a group of biological taxa that includes all descendants of one common ancestor (Merriam-Webster, 1994)) that need to be analyzed and understood. This paper focuses specifically on mammalian herbivores, mammalian predators, and non-human primates (NHP).

By investigating if there is a scientifically significant difference between these partial clades in their changes in stress level and the stressors they are exposed to, researchers can narrow their focus to the types of stressors causing the most harm and can help managers anticipate or eliminate them. This paper looks at the following questions: 1) are anthropogenic stressors the cause of greater stress on an animal vs. non-anthropogenic stressors, and 2) is there a difference between the partial clades indicating one group that has the highest rate in change for their measured FGMs?

Methods

At least seven different studies with a minimum of five species from each group (72 total data points) were analyzed. All of the chosen studies were peer-reviewed research papers published between 2000-2018, ensuring recency. Each study gave measurements of the FGMs either as actual measured amounts, averages or on a graph from which data points could be estimated. Initial papers were found using the MiamiOH library system and Researchgate. For the search criteria the terms "fecal stress captive", "fecal glucocorticoid metabolites animal", "faecal glucocorticoid metabolites" were used, and upon finding a suitable paper, its literature cited section was utilized to detect more studies that could be potential matches. Some studies could have been possible matches, but they could not be analyzed due to access restrictions. Also, if a paper listed data for both wild and captive species, only the data for captive individual's FGM were used. The last search was completed in April 2018.

To determine if there was a scientifically significant difference in the measured FGMs fold change between the three partial clades (herbivores, predators, NHP), 24 peer-reviewed research papers were analyzed. Of the studies done on this topic, there were a variety of mediums surveyed (serum, plasma, saliva, urine, and feces). However, only the hormone concentrations pertaining to fecal hormone levels were focused on due to their higher level of accuracy (feces show an overall average of the FGM that was circulating in the body) and lower impact on the individual animal (Santymire et al., 2012). Most of the data were represented in the reports as an absolute number instead of the fold change; therefore, these data were converted by the equation F=B/A to represent the fold change (Schell et. al, 2013), where F is fold change increase, B is the stressor or post-stressor level of FGM's (ng/g), and A is the baseline FGM (ng/g) level measured prior to the stressor (Fig 1, 2, 3). For the instances where there was a decrease from baseline in FGM concentrations the equation F=(-(A/B)) was used. If the paper gave only the percent change then the percent was divided by 100 to get the fold change (i.e., 186% / 100 = 1.86). If the paper gave a baseline (not average) and a percent change then the baseline was multiplied by the percent to get the peak (i.e., baseline 156 ng with 182% change 156 x 1.82 = 283.92 ng). Once the data were collected per species, the data

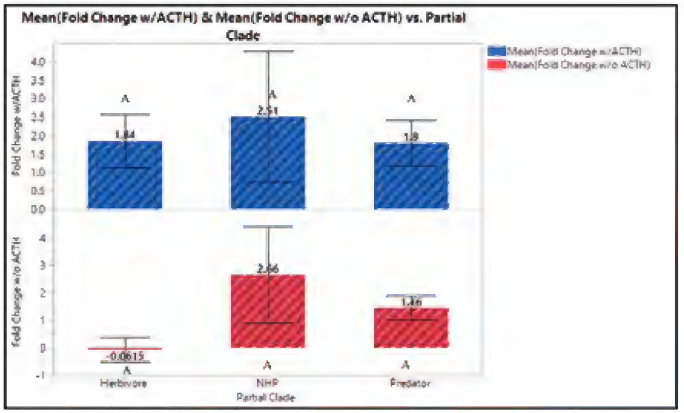

Fig 1: Average fold change between the partial clades for all of the data, including ACTH values: (N: Herbivore = 18, NHP= 22, Predator = 32) and excluding ACTH values: (N: Herbivore = 13, NHP= 20, Predator = 30) With Standard Error bars. Statistical similarities marked by "A".

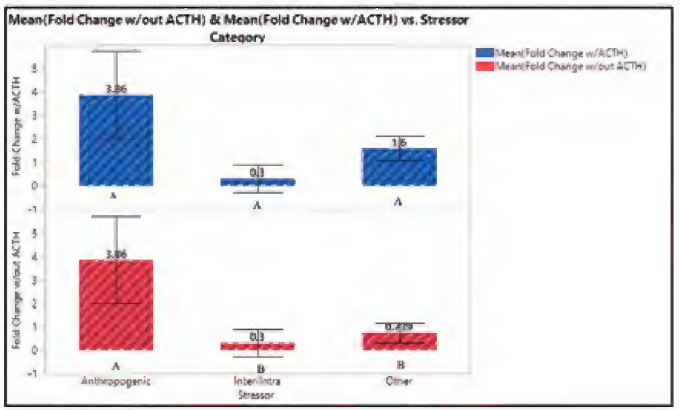

Fig 2: Average fold change between the stressor categories: including ACTH values. (N: Anthropogenic = 18, Intra/Inter Animal = 15, Other = 39), excluding ACTH values. (N: Anthropogenic = 18, Intra/Inter Animal = 15, Other = 30) With Standard Error bars. Statistical similarities marked by "A" and "B".

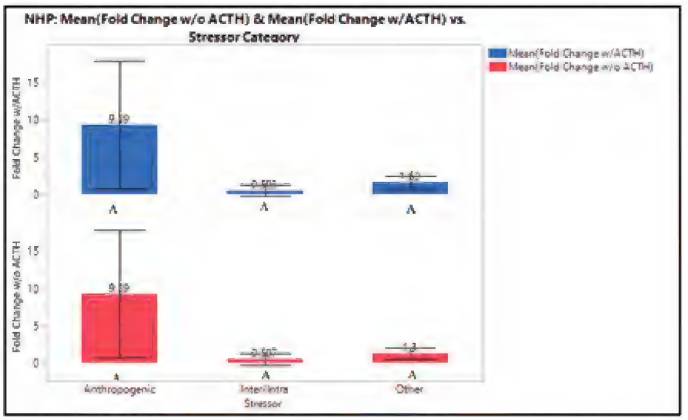

Fig 3: Average fold change between the stressor categories for NHP. Including ACTH values. (N: Anthropogenic = 4, Intra/Inter Animal = 6, Other =12). Excluding ACTH values (N: Anthropogenic = 4, Intra/Inter Animal = 6, Other = 10) With Standard Error bars. Statistical similarities marked by "A".

were combined to find an average fold change value for each partial clade. This was charted to compare each partial clade to the other partial clades in this study (Fig 4).

Each stressor listed in the studies reviewed was marked by color coding as being in one of the three defining categories (Anthropogenic, Inter/Intra Animal, or Other) (Table 1). This was done so that they could be compared against each other to determine if there was a difference in stressor/stress response.

The program JMP 13 was utilized for statistical analysis of the data. A one-way ANOVA and post-hoc Tukey's analysis was performed to test if there is a significant ($P<0.05$) difference between the fold change in measured FGM by partial clade. These analyses were initially run on all of the data for each partial clade separately and then again on all of the same data but excluding data pertaining to the ACTH stimulation injections. This was due to the fact that these injections were an artificial test to ensure the validity of the FGM results. The ANOVA and post-hoc Tukey's tests were applied to the individual partial clades, across all of the partial clades, and across the three categories of stressors per partial clade.

Results

When looking at the data across the three partial clades (Fig 1), there was a positive average fold change when including the ACTH data. Increased across all three partial clades (NHP: 2.7, herbivore: 0.91, predator: 1.9). When excluding the ACTH data, the herbivores showed a negative fold change on average (NHP: 2.66, herbivore: -0.06, predator: 1.46). Once the data were statistically compared across the three partial clades it is clear that there was no significant difference between them ($\alpha = 0.05$: including ACTH: $F = 0.6761$, $P = 0.5119$, and $Q = 2.39532$, Excluding ACTH: $F = 1.2961$, $P = 0.2811$, and $Q = 2.40322$).

The three stressor categories were compared across all three clades with the data that included the ACTH values (Fig 2). There was no significant difference between the stressors for all of the data ($\alpha = 0.05$, $F= 2.4951$, $P = 0.0899$, $Q = 2.39532$). The same tests were run on the data excluding the

ACTH values (Fig 2) and there was found to be a significant difference between the stressors when the one-way ANOVA was run. However, the post-hoc Tukey's analysis did not reveal any significant pairwise comparisons. Therefore, a less conservative Student's post-hoc test was run and a significant difference was found between the Anthropogenic stressors and the inter/intra animal and other stressors ($\alpha = 0.05$, $F = 3.2792$, $P = 0.0445$, $Q = 2.40322$, $t = 2.0003$).

For the NHP (Fig 3), both when including and excluding the ACTH data, there was no significant difference between the stressor categories ($\alpha = 0.05$ Including $F = 2.1686$, $P = 0.1418$, $Q = 2.54045$; Excluding $F = 2.0114$, $P = 0.1644$, $Q = 2.56536$) which means that they were statistically speaking, affected the same no matter what the stressor category.

When looking at the herbivore data, including the ACTH data (Fig 4), there was a significant difference between the stressors of Inter/Intra animal and Other values ($\alpha = 0.05$, $F = 5.5006$, $P = 0.0162$, $Q = 2.59747$). When excluding the ACTH data, there was not a significant difference between the stressors values ($\alpha = 0.05$, $F = 3.5193$, $P = 0.0696$, $Q = 2.74129$). This goes to show how the ACTH data can skew the results, so researchers looking into these comparisons need to be sure to run the data both including and excluding those values.

For the data pertaining to the predators (Fig 5), there was no significant difference between the stressors for the predators found when including the ACTH data ($\alpha = 0.05$, $F = 0.9895$, $P = 0.3840$, $Q = 2.46966$). However, there was a significant difference for the predators between the stressors of Anthropogenic and Other when excluding the ACTH data ($\alpha = 0.05$, $F = 5.1500$, $P = 0.0127$, $Q = 2.47942$). These results show that predators are experiencing more stress due to human-caused stressors.

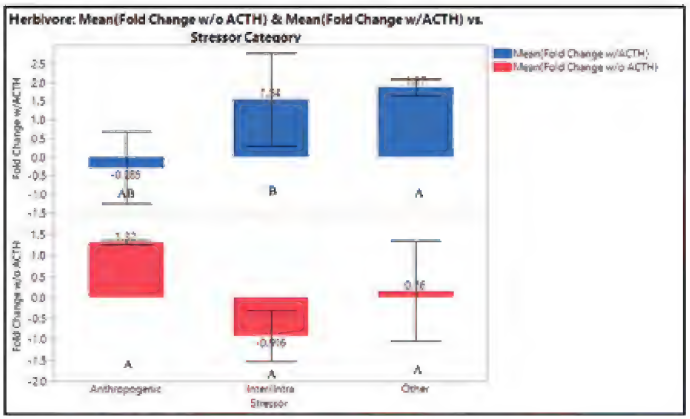

Fig 4: Average fold change between the stressor categories for Herbivores. Including ACTH values. (N: Anthropogenic = 4, Intra/Inter Animal = 7, Other =7). Excluding ACTH values (N: Anthropogenic = 4, Intra/Inter Animal = 7, Other = 2) With Standard Error bars. Statistical similarities marked by "A" and "B".

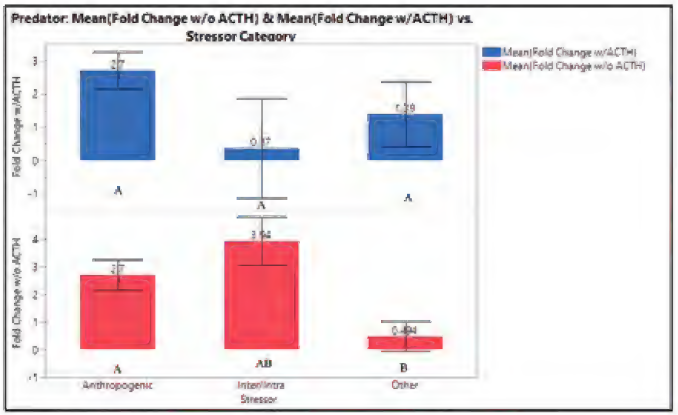

Fig 5: Average fold change between the stressor categories for Predators. Including ACTH values. (N: Anthropogenic = 10, Intra/Inter Animal = 2, Other =20). Excluding ACTH values (N: Anthropogenic = 10, Intra/Inter Animal = 2, Other = 18) With Standard Error bars. Statistical similarities marked by "A" and "B".

Discussion

Through the analysis of three partial clades (NHP, herbivores, and predators) and three stressor categories (anthropogenic, inter/intra animal, and other), this review was able to add a better understanding of captive species and the stressors they are exposed to. From the results the questions posed by this review can be answered: 1. are anthropogenic stressors the cause of greater stress on an animal vs. non-anthropogenic stressors, 2. is there a difference between the partial clades indicating that one group has the highest rate in change for their measured FGMs? Based on this understanding steps can be taken to minimize the negative stressors and their effects.

This review showed that when the artificial stimulation of the HPA axis (ACTH data) is removed from the data for the partial clades there was no difference between the stressors. Yet, when looking at the stressor categories there was a difference that showed that the anthropogenic stressors were causing a greater fold change. This answers the first question that this paper looked to answer (Fig 2).

By comparing the data across the three partial clades (Fig 1), it is easy to see that there was no significant difference between the partial clades in their increase in fold change of measured FGMs. This is demonstrated by the results of the statistical analysis performed on each partial clade separately and then combined. If just the average fold change

is looked at, the NHP is the group that had the largest fold change when the ACTH stimulation data was excluded. The predators had the second largest fold change, with the herbivores instead showing a negative fold change. Further research into the fold change between these partial clades may answer the question of why the NHP would have a larger fold change and the herbivores having a negative one.

Although this report did not reveal a significant difference between the three partial clades responses to stress, it did show that captive animals are experiencing measurable stress between the stressor categories. Due to the evidence presented that they are experiencing stress (Table 2) and possible distress caused by humans, measures need to be taken in order to minimize their exposure to these stressors. Examples of these anthropogenic stressors would be post veterinary procedures in pied tamarins *(Saguinus bicolor bicolo)* (Armstrong and Santymire, 2012) or habitat construction for coyotes *(Canis latrans)* (Ruskell et al., 2015). Clearly, all stressors cannot be removed from a captive animal's life, but more careful and conscious actions can be taken to combat what they are exposed to. Nor are all stressors bad. Many of the studies in this review showed a decrease in FGMs in response to various stressors (Christofolett et al., 2009; Jacobs et al., 2014; Moreira et al., 2007; Liu et al. 2006; Rafac and Santymire, 2013; Zhang et al., 2013). For example, enrichment for Giant Pandas *(Ailuropoda melanoleuca)* (Liu et al., 2006), or enclosure size for Tigrina *(Leopardus tigrinus)*(Moreira et al., 2007) both showed a decrease in average fold changes.

Due to the evidence seen here, we know that the anthropogenic sources are causing the highest levels of change in FGMs. Therefore, we need to be more conscious of those stressors when caring for these species. Perhaps rethinking a construction plan, or the layout of where the visitors are allowed to be in proximity to the animals can minimize the human impact on these animals.

FGMs, as stated by Millspaugh (2004), have the drawback of lacking a large database of species-specific data. Through this review, it is apparent that there is presently more data for predators than for herbivores or NHP. A more comprehensive look into herbivores and NHP is needed to provide greater representation for these findings. Also, many of the studies reviewed had rather small sample sizes of only one or just a few individuals per species. This makes it likely that the species have not been fairly portrayed. A more expansive look at more individuals from each species needs to be completed for these results to be validated. Also, a wider variety of stressors needs to be assessed. This could help avoid bias towards one type of stressor over another. However, a more in-depth look at each stressor will be highly valuable in the future.

There is also the question of whether or not the animals are experiencing distress. An additional study should be conducted in order to find when animals had an increase in FGMs (corticoids, androgens, and progestins) for a sustained period of time and then a drop below baseline levels. This could be an indicator of suppressed gonad function and therefore be an indication of distress (Linklater et al., 2010). It will also be important to start comparing these data to other data collected on the same species in their wild counterparts.

A future project could be implemented across various captive animal facilities, ideally around the world, to gather FGM data on a wide variety of species within each of the three clades assessed in this report. It would be ideal to also have a wide variety of individuals within each species. This would give a much stronger foundation for the results that were seen in this review.

Acknowledgments

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Table 1:
Categorization of the different stressors.

Anthropogenic Stressors Public exposure, habitat construction, veterinary procedures

Inter/Intra-animal Stressors Introduction of a new member, potential mates, removal from group, where they are in the breeding cycle, visibility of predators, housing situation

Other ACTH injection, new habitat, enrichment, transportation, size of enclosure

Table 2 *(opposite page) shows the fold changes measured by species. These changes were calculated via the fold change calculation of F=B/A where F is fold change increase, B is the stressor or post-stressor level of FGM's (ng/g), and A is the baseline FGM (ng/g) level measured prior to the stressor. When B was less than A the equation used was for fold change decrease which is F=A/B. Red = anthropogenic, yellow = intra/inter animal, teal = other. (Armstrong & Santymire, 2012 1; Chosy et al., 2014 2; Christofoletti et al., 2009 3: Dehnhard et al., 2001 4; Jacobs et al., 20145 ; Li et al., 2007 6; Liu et al., 20067 ; Loeding et al., 20118 ; Moreira et al., 20079 ; Narayan et al., 2013 10 ; Rafac & Santymire, 201311 ; Rimbach et al., 201312 ; Ruskell et al., 201513 ; Santymire et al., 2012 14 ; Schell et al., 201315 ; Shepherdson et al., 201316; Sherwen et al., 201517; Takeshita et al., 201418; Webster et al., 201619 ; Weingrill et al., 201120; Wielebnowski et al., 200221 ; Young et al., 201722; Zaragoza et al., 201123; Zhang et al., 201324) * = the data was estimated off of a graph.*

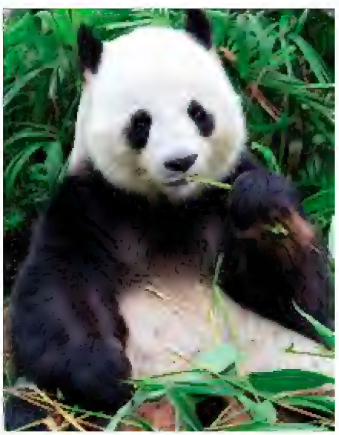

Table 2: Stressors per Partial Clade Mean Fold Increase of FGMs

Stressor	Non-Human Primates	Herbivores	Predators
ACTH Injection	Chimpanzee *(Pan troglodytes)* 14: 1.24, Vervet monkey *(Chlorocebus pygerythrus)* 22 ; 4.95	Black Rhino *(Diceros bicornis)* 14: 1.65, African Elephant (Loxodonta africana) 14: 2.09, Roe Deer *(Capreolus capreolus)* 4: 7.32 , Koala *(Phascolarctos cinereus)* 10: 1.86, Brown Brocket Deer *(Mazama gouazoubira)* 3 : 4.25	Coyotes *(Canis latrans)* 15: 10.75, African Lion *(Panthera leo krugeri)* 14: 1.56
New Habitat / enclosure	Brown Spider Monkey *(Ateles hybridus)* 12: -2.59		
Red Howler Monkey (Alouatta seniculus)12: 2.38	Père David's Deer *(Elaphurus davidianus)* 6 : 1.36 *	Afghan leopard *(Panthera pardus saxicolor)* 2: 1.48, Black leopard *(Panthera pardu)* 2: -1.10, Serval (*Leptailurus serval)* 2: 1.84, Snow leopard *(Uncia uncia)* 2: -1.02	
Enrichment (During)	Chimpanzee *(Pan troglodytes)* 23 ; 1.14, Western Gorilla *(Gorilla gorilla)* 23; 1.82		African Wild Dog *(Lycaon pictus)* 11: -1.02, Bengal tigers *(Panthera tigris tigris)* 13; -3.28, Cougar (*Felis concolor)* 13; -1.64
Enrichment Addition (post)		Giant Panda *(Ailuropoda melanoleuca)* 7 : -1.04*	African Wild Dog *(Lycaon pictus)* 11: -1.17, Bengal tigers *(Panthera tigris tigris)* 13, 2.22, Cougar *(Felis concolor)* 13; 1.76
Transportation	Orangutans *(Pongo pygmaeus)* 20 : 2.54 *, Brown Spider Monkey *(Ateles hybridus)* 12: 3.45		
Red Howler Monkey (Alouatta seniculus) 12: 4.22		Polar Bear *(Ursus maritimus)* 16: 6.99	
Post Transportation	Orangutans *(Pongo pygmaeus)* 20 0.2*		Polar Bear *(Ursus maritimus)* 16: 1.64
Large Enriched Enclosure			Tigrina *(Leopardus tigrinus)* 9 : -1.07* , Margay *(Leopardus wiedii)* 9 : -1.3*
Small Barren Enclosure			Tigrina *(Leopardus tigrinus)* 9 : 1.45* , Margay *(Leopardus wiedii)* 9 : 1.12*
Small Enriched Enclosure			Tigrina *(Leopardus tigrinus)* 9 : -1.12* , Margay *(Leopardus wiedii)* 9 : -0.54*
Intro to a new group		Sable *(Hippotragus niger)* 8:1.47	
Post Intro to a new group		Sable *(Hippotragus niger)* 8:1.47	
Pre-Intro to a mate	Lowland Gorilla *(Gorilla gorilla gorilla)* 5:1.62		
Intro to a mate	Lowland Gorilla *(Gorilla gorilla gorilla)* 5:1.15		
Post Intro to mate	Lowland Gorilla *(Gorilla gorilla gorilla)* 5: -1.58		
Predators Visible			Cloud Leopard *(Neofelis nebulosa)* 21: 3.06
Predators not Visible			N.A. Cloud Leopard 21 ; 4.81
Removal From Group	Golden Snub-Nosed Monkeys *(Rhinopithecus roxellana)* 24: -1.52 ; Japanese Macaquas *(Macaca fuscata)* 18: 2.38*		
Group mate removed	Golden Snub-Nosed Monkeys *(Rhinopithecus roxellana)* 24: 1.38		
Mating to Birthing Season	Japanese Macaquas *(Macaca fuscata)* 18:0.6*		
Estrus		Giant Panda *(Ailuropoda melanoleuca)* 7 : 1.21*	
Post - Estrus		Giant Panda *(Ailuropoda melanoleuca)* 7 :1.42*	
Housed Together: Outside Exhibit		Brocket Deer *(Mazama gouazoubira)* 3 : -1.21	
Housed Individually		Brocket Deer *(Mazama gouazoubira)* 3 : -2.56	
Housed Together Outside Exhibit Day, Individual Night		Brocket Deer *(Mazama gouazoubira)* 3 : -2.33	
Habitat Construction			Coyotes *(Canis latrans)* 15: 4.25, Afgan leopard *(Panthera pardus saxicolor)* 2: 1.39, Black leopard *(Panthera pardu)* 2: 1.05, Serval *(Leptailurus serval)* 2: 1.94, Snow leopard *(Uncia uncia)* 2: 1.01
Post- Vet Procedure	Pied Tamarins *(Saguinus bicolor bicolo)* 1: 18.29	Sable *(Hippotragus niger)* 8: 1.3	Bengal tigers *(Panthera tigris tigris)* 13; 5.6, Cougar *(Felis concolor)* 13; 1.19
Public Exposure: Standard		Koala *(Phascolarctos cinereus)* 19: 1.38, Père David's Deer *(Elaphurus davidianus)* 6 : 1.21 *	Coyotes *(Canis latrans)* 15: 5.17, Cloud Leopard *(Neofelis nebulosa)* 21: 2.65
Public Exposure: Intense		Koala *(Phascolarctos cinereus)* 19: 1.40	
No/diminished Public Exposure	Black Capped Capuchin *(Cebus apella)* 17 ; -1.07		N.A. Cloud Leopard 21: 2.78

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No Tall Tale: The silent plight of giraffe

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GPS satellite tracking unit mounted to the ossicone. Photo by K Bohn SDZG/GCF

Giraffe *(Giraffa camelopardalis)*, one of the world's most iconic animals, may soon be joining the endangered species list. Many people don't realize there are four different species of giraffe with five subspecies, four of which are already considered endangered or critically endangered with risk of extinction. This beloved giant is well equipped to handle lions and hyenas, but comes up shorthanded when faced with habitat loss and poaching (Fennessy, Bidon, Reuss, Kumar, Elkan, Nilsson, Vamberger, Fritz, Janke, 2016).

It's estimated that over 30% of wild giraffe populations have been lost since the 1990s with minimal commentary (Fennessy, 2019). We're familiar with struggles of poaching rhinos for horns,

big cats for pelts, and elephants for ivory: but what is the giraffe's story? Why are they disappearing and how can you help?

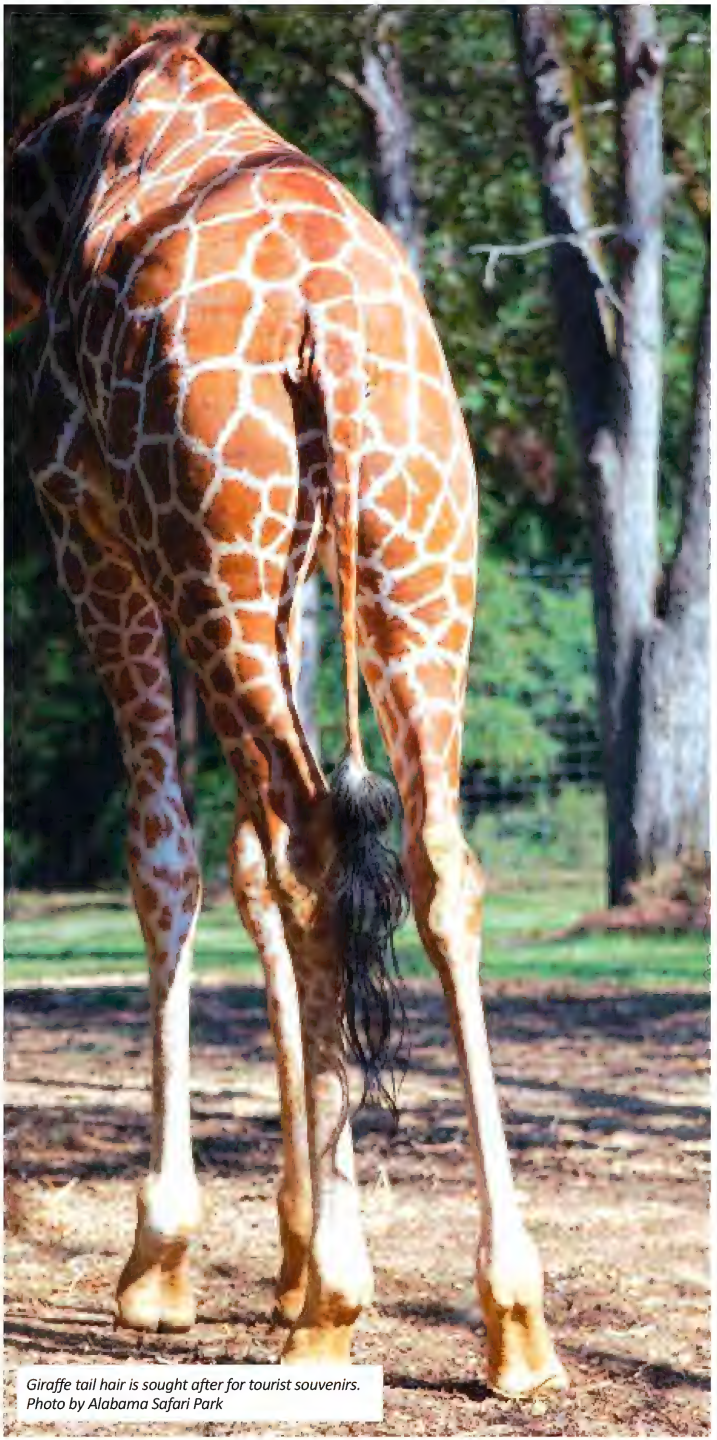
Giraffe populations struggle due to habitat loss, habitat fragmentation, and poaching. Illegal hunting in Tanzania has impacted giraffe populations due to a misinformed new belief that giraffe brain and bone marrow can cure HIV-AIDS. There is no scientific data that supports this theory. Fresh giraffe head and bones can fetch prices up to $140 per piece (Muller, 2010).

In the Congo, poachers are interested in a different body part: the tail. African Parks' Joint Operations Director Leon Lamprecht explains the motivation behind this unusual hunt, in that local Congolese men use the tail as a marriage dowry to the bride's father (Lamprecht, 2016).

Local traditions are not the only reason for the demand of giraffe tail. Tourists may be unknowingly purchasing souvenirs made out of the long black hairs such as good-luck bracelets, fly whisks, and thread for sewing or stringing beads. Most of these products originate from South Africa where Giraffe products can be sold legally. (Muller, 2010).

It's no wonder saving giraffes seems like such a daunting task. Giraffe currently occur in 21 African countries facing different threats that need a wide range of conservation approaches to secure their future. Luckily, zoos around the world have come together to give voice to this silent extinction. Friday, June 21st was World Giraffe Day, 2019 and giraffe lovers around the world came together with a goal to raise $1 million for the Giraffe Conservation Foundation's (GCF) project known as the "Twiga Tracker." Twiga is Swahili for giraffe. The Twiga Tracker project is the largest giraffe GPS satellite tracking program ever conducted in Africa, with a goal to track a minimum of 250 wild giraffe to better understand their behavior and the challenges they're facing. Each specialized tracking unit costs $2,500 and the cost to deploy these units in the field is estimated at an additional $5,000.

In an effort to save giraffe, the Virginia Safari Park, Gulf Breeze Zoo, and Alabama Safari Park joined the World Giraffe Day Celebration. The weeklong celebration



Giraffe tail hair is sought after for tourist souvenirs. Photo by Alabama Safari Park

included behind the scenes tours, conservation-centered keeper talks, as well as a 10% donation for all giraffe giftshop merchandise sold. Guests were surrounded all week by everything giraffe. They learned about husbandry care for captive animals, as well as the conservation issues facing wild populations. Tours provided a behind the scenes look at giraffe holding facilities, a custom designed giraffe transport trailer, and a one-on-one close-up feeding experience with the resident giraffe herd. The event was successful with 165 behind the scenes giraffe encounters conducted throughout the parks. A total of $5,000 was raised for the Twiga Tracker program.

Programs like these can only succeed when people are committed on a global scale. The Virginia Safari Park, Gulf Breeze Zoo, and the Alabama Safari Park are excited to partner with the Giraffe Conservation Foundation to help with their continued success. Our animal ambassadors play an important role in giving a voice to their wild counterparts by raising awareness and offering financial support. The issues facing wild giraffe are intimidating, yet it is important to keep in mind that, while there is life, there is hope. We will continue to support one another in this fight to save this gentle giant, giraffe.

Feeding giraffe

Guests engaging with giraffe during a World Giraffe Day Tour. Photo by Gulf Breeze Zoo

Our animal ambassadors play an important role in giving a voice to their wild counterparts by raising awareness and offering financial support.

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Injection Training with a Male Western Lowland Gorilla

Kirsten Everett – Senior Zoo Keeper
Zoo New England's Franklin Park Zoo, Boston, MA

Kit. Photo by Christina Demetrio

INTRODUCTION

Kitombe, "Kit" for short, is a 31-year-old male Western Lowland Gorilla (*Gorilla gorilla gorilla*) residing at Zoo New England's (ZNE) Franklin Park Zoo (FPZ) in Boston, Massachusetts. He was born at the Audubon Zoo in New Orleans, Louisiana and moved to FPZ in 1998 to be the breeding male in our troop.

Kit has sired five daughters, his first in 1996 at Audubon Zoo; the rest at FPZ, including his most recent in 2015. As of October 2018, our troop consisted of five females, Gigi, 46; Kiki, 37; Kimani, 13.5; Kambiri, 7.5; Azize, 3, and our two males, Kit, 31.5 and Joe, 25. Kit and Kiki continue to be our breeding pair. The three youngest females, Kimani, Kambiri and Azize are their offspring.

ZNE has established behavioral conditioning programs for our animal collection to facilitate medical procedures and general care of the animals. Operant conditioning techniques are used and all animal participation is voluntary. In our gorilla program we have trained a variety of behaviors: blood pressure, cardiac ultrasound, injection, and basic behaviors for doing thorough body visual exams.

CHALLENGES

I have been caring for Kit for nine years and have been his primary trainer since 2014. Unlike most of the other gorillas that I work with, Kit is not especially interested in interacting with his human caretakers and tends to be more focused on his gorilla family. Kit also never solicits keepers' attention in the off-exhibit space or while on exhibit.

When Kit is off-exhibit, he prefers to have a visual of his family. Even when he is given the entire off-

exhibit area, he always chooses to sit by the exhibit entrance so he can maintain this visual. This part of his personality makes training difficult because he is not motivated to move away from this area and it's not the best place to access him for training.

When working with Kit one on one, he appears relaxed. When multiple keepers or guests observe a training session, he gives the impression that he is not comfortable. He is easily distracted by any unexpected movement they may make and responds to this by barking and dividing his focus between the trainer and the observers.

Kit is blind in his right eye and is easily startled. He does not present the right side of his body well. He also appears to act suspicious of what his keepers are asking him to do or where we ask him to go. For example, he takes time to peer in and around the den he is asked to move into for training and will sometimes break station to look around at his surroundings, even if nothing has changed. It's possible that his blindness causes him to be insecure about his surroundings.

Another distraction during training sessions are his issues with constipation. Kit would walk away from a training session if he had to have a bowel movement. This has since been resolved with the addition of MiraLAX® to his diet, but he continues to leave to urinate, which interrupts the flow of a session. It is for these reasons that I have found working with Kit to be quite the challenge.

Needle props

TRAINING

In general, food is Kit's only motivation to interact with his keeper staff. For this reason, I must use high value food items to encourage his participation during training sessions. Reinforcements such as fruits, nuts, or juice keep his attention. Despite his general lack of interest in training, Kit has learned many behaviors over the years with different trainers: open mouth, ear, arm, shoulder, foot, back, chest, belly, chin, brow, knee, and hip.

Injection History

Kit has always been very averse to receiving injections. When he sees a syringe, or any object that looks similar, he recoils and holds his arm up like a shield. Despite this, he must receive a flu vaccine annually. Traditionally, vaccines have been given to him when he's distracted. For example, during feeding, a keeper would sneak an injection into his upper arm while another keeper kept his attention with rewards.

Previous trainers and I spent a lot of time trying to desensitize him to a needle and syringe. Over time, we got very creative with the types of props used to shape the injection behavior due to his apparent dislike of the syringe and needle. At first, trainers tried many different sized syringes with no needle. Once Kit would sit for a training session without flinching at the sight of a plain syringe, a wooden end of a cotton swab was then added as a fake "needle". Syringes were even disguised using different colors of vet wrap to help desensitize him to its look or to hide the liquid medication within. Initially, each time a syringe or syringe and needle was picked up from his training box he would flinch. During these sessions, he was rewarded if the trainer could touch a syringe or needle that was in the box. Then we rewarded him if he didn't flinch when we took the syringe out of the box and put it on the floor next to us. Our next step was to put the syringe in our lap without him flinching. Moving forward we would then try holding the syringe in our hand without him flinching. We then worked up to touching his foot and then, gradually, his arm. During these sessions, Kit would make progress and allowed us to touch him. Then, inexplicably, something would change for him and he would not accept the presence or being touched by any of the syringe props. Then we would have to start from the beginning again. We tried this method many times over, but none of these efforts helped him to accept a needle and syringe. The most progress I made with him was to touch his upper arm with a capped needle, but it never seemed to get easier for him.

In 2016, I gave him his flu vaccine for the first time. In the fashion I had learned from past trainers, I hid the needle, asked for his right ear and while he leaned in close to the mesh, I took the needle from out of his view and injected him in his upper arm. Immediately, he backed up and held his arm, touched his fingers to the injection site, smelled his fingers, and roared and banged on the mesh. At this point, I realized that "tricking" him into accepting a vaccine was impacting our training relationship in a negative way

and that Kit may not ever voluntarily accept an injection in his upper arm.

A New Approach
My idea was to get rid of the sight of needles all together and change the injection site to his hip area. If I could station him against the mesh while he was being rewarded by another keeper, I would have access to his side and hip and could work on desensitizing him to an injection in this fleshy area. My thought was that the "feeling" of an injection may not be as stressful to Kit as seeing the actual needle.

With the help of my fellow keepers, I started this new approach. The second keeper would touch his shoulder with a tongue depressor after I gave Kit the shoulder cue. Kit's first reaction was to bark at the second keeper when they touched him. He acts suspicious of training sessions in general and this was heightened when two keepers were present. It took many sessions for him to participate without barking. In the end, I would bridge and reward him for accepting a touch from the second keeper without protest.

Training Plan – Break it down! Below is a list of approximations used to train the new hip injection behavior:

1. Introduce a second keeper sitting next to the trainer – we will refer to he/she as the secondary and to myself as the primary.
2. Primary gives Kit a cue such as "arm" and secondary touches Kit's arm with a tongue depressor.
3. Primary bridges and rewards Kit for allowing secondary to touch his arm.
4. Once Kit is comfortable with having a second person involved, move secondary's position to inside the adjacent den with his food reinforcement (Image 2).
5. Primary will ask for a behavior and bridge for compliance. Primary points to secondary in the adjacent den, requiring Kit to turn 90 degrees to receive his food reward from the secondary.
6. After Kit is rewarded, primary calls Kit to station (sit facing primary) and cues him to "brace" which is to hold onto the mesh above his shoulder height with both hands. Repeat as needed until he understands that the reward will be given in this manner.
7. Primary cues the "hip" behavior, Kit's position is standing on all fours facing secondary with hip pressed against mesh closest to the primary. Secondary rewards continuously while his hip is pressed against the mesh. If he pulls his hip away from mesh, reinforcement stops. Primary cues "hip" again and reinforcement resumes when he pushes his hip against mesh again.
8. Once he presents his hip consistently, primary begins to touch hip with ¼" dowel as long as a pencil.
9. Once comfortable with being touched with the dowel, increase the amount of pressure and create a mild sensation by gently pulling on tufts of hair on his rear, hip, shoulder and arm while he is standing for reinforcement. A verbal bridge "good" and cue of "hold" are used to increase the duration in this position.
10. Begin applying pressure with a sharper object, in this case a wooden end of a 6" cotton tipped swab.
11. Primary increases pressure until he would no longer tolerate it, break position and bark at the primary trainer. This was done initially to know the maximum amount of pressure to give before he reacts to it, with the goal being to bridge and reward before reaching the discomfort threshold.

12. When it's time for the actual injection: Position the needle and syringe out of view and give the cue for hip. Immediately before injecting, create sensations in the area by pulling on tufts of hair or flicking a finger on the skin. Swiftly give the injection in hip without him seeing the needle or syringe. Following injection, it is critical to keep the needle and syringe out of view. Bridge and reward with favorite treats.

Giving the First Injection

Following step 12 of our behavioral plan, I asked for hip and gave him a few pokes with the cotton swab stick while the secondary trainer consistently rewarded him. Then I pulled the flu vaccine out of his training box (out of sight) and carefully, but swiftly, injected him in the muscle of his hip. I then put the needle away in my training box before he could see it. He pulled away to see what just happened so I showed him my swab stick and asked him to press his hip against the mesh again. He did! He did not touch the site of injection this time. He held his hip against the mesh for constant rewards from the secondary trainer and I ended the session with verbal praise and a banana.

Juice bottle

I was very pleased with Kit's reaction to the injection. I was eager to train him soon after to see if he would give me hip again without reservations. We did a training session two days later in the same manor and he did not have any adverse reaction to me asking for the hip behavior. He performed the behavior just fine, and was rewarded with his favorite treats.

CONCLUSION

Sometimes you have to think outside the box and change your normal training approach in order to achieve your goal. Instead of training by myself I added a second keeper and together we were able to utilize the holding space differently than before. Providing continuous food reinforcement was also key. Kit was able to be rewarded constantly throughout the sessions while I worked on desensitizing the side of his body. Kit doesn't need to see the needle, he just had to learn to present his body in a new way and tolerate the sensation. Your fellow keepers are a great resource. I have to thank all the keeper staff that have given their time and patience to help me train Kit. Their assistance helped ease the difficult task of injecting a needle-averse male gorilla. I will continue this training specifically for the flu vaccination with Kit. This injection training will be helpful for future challenges such as additional vaccinations or even immobilization medication. Every five years Kit needs a full physical exam, which requires him to be anesthetized. This injection of anesthetics requires a larger volume of drugs with a larger gauge needle. For his last exam, our vet staff darted Kit with the anesthetics, which is very stressful. It would be less stressful for him if I was able to hand inject him instead of being darted. This is going to take time and patience for two reasons. It is a more sensitive injection and the off-exhibit space where these exams take place is laid out differently than where Kit is accustomed to receiving his flu shot. Hopefully, with the innovative training I used to accomplish his flu vaccine, I can be successful in helping Kit overcome ANY injection.

ACKNOWLEDGEMENTS

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Training Tales Editorial

Angela Binney
Training Tales
Column Co-Coordinator

There truly is no one-size-fits-all training plan. Considering individual history is crucial to addressing challenges. Though consistency is useful when developing a program for a group of animals and keepers, it is important to customize plans to meet individual needs when necessary. This can sometimes mean taking a completely different approach in order to overcome established negative associations. In other words, un-training is so much harder than training.

I love the way the trainer continued asking for behaviors after the injection had been accomplished as if nothing unusual happened. This goes against the inclination to end immediately after the successful injection with a huge jackpot and lots of praise. End on a high note, right? For Kit, a large celebrated ending at that point could draw attention to the fact that he just got tricked, once again, into receiving the very thing he had worked so hard to avoid in the past. Even if he noticed the sensation of the vaccine (which is highly likely), he was hopefully distracted and possibly comforted by the appearance of calm normalcy. The last thing he experienced during the session is not that odd tingly sensation he felt part way through, but the positive reinforcement received after a few easy behaviors paired with a calm peaceful interaction with his trainers.

Thank you for sharing your Training Tale. I wish you continued success as you approximate toward hand injecting for anesthesia!

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